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TREATMENT OF XANTHOMONAS HYDROPHILIC COLLOID AND RESULTING PRODUCT

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15 Claims

ABSTRACT OF THE DISCLOSURE

Process for purifying a *Xanthomonas* hydrophilic colloid containing proteinaceous impurities in aqueous media by maintaining the pH of the media above about 8.0, preferably above about 10.0, adding an alkali metal hypochlorite to the mixture, preferably at a concentration of about 0.25 to 2.0 percent, then adjusting the pH of the media to slightly acidic, and adding a lower alcohol to precipitate the *Xanthomonas* hydrophilic colloid.

This invention relates to an improved hydrophilic colloid obtained from treatment of the colloid produced by a *Xanthomonas* bacteria. More particularly, the present invention pertains to a method for improving the clarity of a *Xanthomonas* hydrophilic colloid and also to the colloid so obtained.

Xanthomonas hydrophilic colloid is a biosynthetic polysaccharide composed of glucose, mannose, and glucuronic acid in the molar ratio 2:1:1, with approximately each $\frac{1}{4}$ unit of polymer containing one acetyl group. It disperses or dissolves in water to give solutions of greatly increased viscosity, and in that respect is similar to some other natural gums. However, the clarity of such solutions is not very great, and the general opacity of the solutions combined with an off-white to yellowish tinge makes a *Xanthomonas* hydrophilic colloid, as ordinarily obtained, unsuitable for many applications. Furthermore, the colloid as ordinarily obtained is unduly subject to spoilage when in aqueous solution and it is necessary to incorporate chemical preservatives such as chlorinated phenolic compounds, formaldehyde, and the like. It appears that the spoilage tendencies of the *Xanthomonas* hydrophilic colloid are associated with the components in the colloid, as ordinarily produced, which cause opacity and off-white coloring of its solutions.

A previous process for the clarification of a *Xanthomonas* hydrophilic colloid was based, at least to some degree, on the use of filtration. Due to the difficulties involved in filtering a *Xanthomonas* hydrophilic colloid, which is a relatively thick viscous material, the previous process required that the *Xanthomonas* hydrophilic colloid be diluted and also required that it be heated and cooled under controlled pH conditions prior to filtration.

The previous process is relatively time consuming and costly because of the requirement that the *Xanthomonas* colloid be diluted and filtered. Thus, an object of the present invention is to provide a process for purification of a *Xanthomonas* hydrophilic colloid which does not require dilution of a *Xanthomonas* hydrophilic colloid coupled with filtration as a means of obtaining a material having improved clarity and stability.

Additional objects will become apparent from a reading of the specification and claims which follow:

As a starting material, I employ a *Xanthomonas* hydrophilic colloid solution and preferably a crude solution thereof known as a beer. A *Xanthomonas* hydrophilic colloid that is particularly suitable for use in accordance with my invention is such a colloid prepared by the bacterium *Xanthomonas campestris*. The said colloid is a polymer containing mannose, glucose, and potassium glucuronate. In such a colloid, the potassium portion can

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be replaced by several other cations without substantial change in the property of the said material for my purpose. The said colloid, which is a high molecular weight, exocellular material, may be prepared by the bacterium *Xanthomonas campestris*, by whole culture fermentation of a medium containing 2–5 percent commercial glucose, an organic nitrogen source, dipotassium hydrogen phosphate and appropriate trace elements. The incubation time of the final medium is approximately 96 hours at 30° C. under aerobic conditions. In preparing the colloid as aforesaid, it is convenient to use corn steep liquor or distillers' dry solubles as an organic nitrogen source. It is expedient to grow the culture in two intermediate stages prior to the final inoculation in order to encourage vigorous growth of the bacteria. These stages may be carried out in media having a pH of about 7. In a first stage a transfer from an agar slant to a dilute glucose broth may be made and the bacteria cultured for 24 hours under vigorous agitation and aeration at a temperature of about 30° C. The culture so produced may then be used to inoculate a higher glucose (3%) content broth of larger volume in a second intermediate stage. In this stage the reaction may be permitted to continue for 24 hours under the same conditions as the first stage. The culture so acclimated for use with glucose by the aforementioned first and second stages is then added to the final glucose medium. In the aforesaid method of preparing *Xanthomonas campestris* hydrophilic colloid, a loopful of organism from the agar slant is adequate for the first stage comprising 200 milliliters of the said glucose medium. In the second stage the material resulting from the first stage may be used together with 9 times its volume of a 3% glucose medium. In the final stage the material produced in the second stage may be admixed with 19 times its volume of the final medium. A good final medium may contain 3% glucose, 0.5% distillers' dry solubles, 0.5% dipotassium phosphate, 0.1% magnesium sulphate having 7 molecules of water of crystallization and water. The reaction in the final stage may be satisfactorily carried out for 96 hours at 30° C. with vigorous agitation and aeration. A *Xanthomonas* colloidal solution in the aforementioned stage of preparation is known as a beer. This is a preferred starting material for practicing my invention and, i.e., the beer resulting directly from the said fermentation may be used as the starting material.

In practicing my process, a fermentation beer containing a *Xanthomonas* hydrophilic colloid, which may be prepared in the manner described above, is treated with a selected oxidizing agent. Although the starting material is preferably a fermentation beer, my process may also be applied to solutions of a *Xanthomonas* hydrophilic colloid. After treatment of the fermentation beer with the selected oxidizing agent, the *Xanthomonas* hydrophilic colloid is readily separated from the impurities therein through precipitation of the colloid in alcohol.

Surprisingly, the specific oxidizing agent which is employed in my process is quite critical to the success of the process. The oxidizing agents which I have found suitable are the alkali metal hypochlorites, such as sodium hypochlorite, potassium hypochlorite, and lithium hypochlorite. Seemingly closely related materials, such as hydrogen peroxide, and chlorine gas do not function in my process.

The alkali metal hypochlorite may be added to the fermentation beer, as described above, or it may be formed in situ by the addition of chlorine gas with an alkali metal hydroxide, e.g., sodium hydroxide, which materials react to form the alkali metal hypochlorite, e.g., sodium hypochlorite. During the addition of the selected oxidizing agent, the pH of the reaction medium containing the *Xanthomonas* hydrophilic colloid fermentation beer is maintained above about 8.0 and more preferably is main-